

Sleep Disruption in a Mouse Model of Medial Temporal Lobe Epilepsy

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Lucie Rosenberg

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Sleep Disruption in a Mouse Model of Medial Temporal Lobe Epilepsy

Approved by:

Dr. Nigel Pedersen

Department of Neurology

Emory University School of Medicine

School of Biomedical Engineering

Georgia Institute of Technology

Dr. Anabelle Singer

School of Biomedical Engineering

Georgia Institute of Technology

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Abstract

While not typically a recognized comorbidity of epilepsy, sleep disorders are common in epilepsy, particularly medial temporal lobe epilepsy (MTLE). However, consistent and severe sleep disruptions have a potentially cascading effect in the severity of seizure control, as well as other comorbidities of TLE. The present study uses the intra-amygdala kainic acid (IAKA) mouse model of TLE to develop a basis for novel sleep-focused treatments. Using a custom 3-D printed headset equipped with electroencephalogram (EEG) electrodes, electromyogram (EMG) electrodes, and a guide cannula, mice were injected with kainic acid targeting the left basolateral amygdala and were recorded continuously throughout the duration of the experiment. Preliminary results show that IAKA mice (with seizures) spent more time in wake and less time in non-rapid eye movement (NREM) and rapid eye movement (REM) sleep over the experiment's final 72 hours, compared to controls. Furthermore, a weak positive correlation was identified between percent of time spent in wake and amount of seizures. Histological data showed amygdala and hippocampal damage in mice with seizures.

Introduction

Temporal lobe epilepsy (TLE) is the most prevalent and drug-resistant form of adult epilepsy (Zenteno et al. 2012), with the bulk of these patients having medial TLE (MTLE). Even among patients with seizures regulated by medications, side-effects are common. About a third of patients with TLE do not respond well to anti-epileptic drugs (Granata et al. 2009, Kwan & Brodie 2000) and only approximately 32-75% of patients experience seizure freedom after surgical treatment of their epilepsy (Téllez-Zeteno et al. 2010). The epileptogenic focus in MTLE lies in the hippocampus and surrounding structures, and the progressive pathology is correlated with memory disorder in humans, possibly related to various comorbidities such as

depression, anxiety, impaired cognitive performance, and spatial memory deficits (Grötcke et al. 2008). Some of the lesser investigated, yet pronounced, candidate comorbidities in epilepsy are sleep disturbances, including insomnia which is characterized by difficulty initiating or maintaining sleep (Cormier, 1990). Insomnia is 2-3 times higher in people with epilepsy (Latrielle et al., 2018, Macedo et al., 2017) and seen as a contributing factor to poor seizure control among patients (Spencer et al., 2016). It can be proposed that insomnia likely contributes to the severity of a patient's epilepsy, as well as potentially worsening other comorbidities.

Sleep and epilepsy are the topics of two significant bodies of science that have become divergent. The relationship between sleep and epilepsy has been acknowledged since the time of Aristotle and Hippocrates, but has not been investigated mechanistically, with available literature being limited to descriptive, observational or purely clinical perspectives. Sleep literature has a primary focus in sleep disorders such as sleep apnea, along with a significant body of work about sleep-wake mechanisms. On the other hand, epilepsy literature describes local circuitry of epilepsy, often in relation to clinical applications.

It is important to investigate the application of the sleep-wake circuitry, consisting of the hypothalamus, preoptic nuclei, basal forebrain, and upper brainstem, and its influence on cortical rhythms and excitability (Saper et al. 2017). This influence over cortical rhythmicity and excitability must contribute to seizure risk and severity, as evidenced by most patients' habitual relationship of seizures to their sleep-wake cycle or a particular sleep-wake state. This relationship – the sleep-wake disruptions in response to seizure activity, as well as the potential for manipulations of sleep-wake circuits to control seizure – is the backdrop for the proposed studies.

The intra-amygdala kainic acid (IAKA) rodent model of MTLE has yielded a strong analogue to behavioral and electrographic seizures in human patients, mirroring human histopathology and comorbidities (Akari et al., 2002). Notably, our preliminary data shows that the IAKA model of epilepsy produces similar sleep disturbances in mice as those reported by patients. Investigation of these sleep disturbances and their relationship to seizure frequency and intensity provides a novel way to bridge the gap in the literature, as well as providing necessary background for other experiments that will address mechanisms of seizure threshold and pave ways for new potential treatments.

The present study aims to establish a model of MTLE that focuses on the sleep disruptions coupled with epilepsy in mice. In order to establish a comprehensive model, the present study will first investigate a useful transgenic strain of mice, known to have a lower seizure threshold to electrical kindling, and later compare to wild-type mice to highlight any potential differences in seizure threshold or severity of epilepsy resulting from IAKA (Straub et al. 2020). There is a strong reciprocal relationship between sleep and epilepsy, meaning that the more a patient or animal is sleep deprived, the higher their propensity for seizures. By administering injections of kainic acid into or surrounding the targeted basolateral amygdala, mice undergo status epilepticus (SE) and subsequently develop spontaneous seizures, which we propose will result in fragmented sleep. Thus, by establishing a strong model of sleep disorders in epilepsy, seizures and other comorbidities of epilepsy can be investigated via novel interventions.

Literature Review

The present study aims to establish a sleep-centered IAKA mouse model to serve as the background for developing novel interventions to regulate MTLE seizures via finely tuned

manipulation of the sleep-wake circuits. While epilepsy is commonly defined as a disorder of excessive synchrony and excitability within the cerebral cortex (Worrell et al. 2000), the observation that both - synchrony and excitability - are controlled by subcortical sleep-wake circuits (Saper et al. 2010) is underappreciated in the epilepsy literature. TLE often has a habitual relationship to sleep-wake states, with seizures typically occurring preferentially in wake for some patients and in sleep for others, rather than sporadically (Crespel et al. 2000). These findings further support the existence of a relationship between MTLE and sleep, as well as providing a possible explanation for the nature of the relationship.

The understanding of the bidirectional nature of sleep and MTLE is crucial to the translational nature of this study. The reciprocal nature between the effects of sleep deprivation as it relates to epilepsy can create a dangerous spiral effect for individuals with epilepsy. Patients with TLE often exhibit a preferential occurrence of seizures during daytime and wakefulness, in addition to sleep fragmentation, characterized by repetitive short interruptions of sleep (Smurra et al. 2001), and it is common for TLE patients to experience more seizures during a state of sleep deprivation, particularly resulting in convulsions upon awakening (Rajna & Veres, 1993). In stage II non-REM sleep, the synchronizing effects of thalamic circuits may underlie the facilitation of interictal abnormalities, contributing to the propagation of seizures during sleep stages (Crespel et al. 2000). As shown by Ng and Pavlova in their 2013 study where they pooled temporal and extratemporal epilepsy, when focal seizures do occur in sleep, they are typically during the first two stages of non-REM sleep, with the lowest occurrence during REM and deep sleep.

Much like the general body of sleep and epilepsy literature, the clinical applications of research regarding sleep and epilepsy are also historically divided, with each field having its own

extensive body of literature but with those bodies being mutually exclusive (Dement et al., 1998). Historically, sleep research has been focused on obstructive sleep apnea, only recently shifting to focus on investigation of the physiology of sleep and effects of sleep deprivation. Sleep and epilepsy each have focused on mechanistic and circuit-based approaches, with epilepsy research highlighting a circuits-based approach, typically only within local circuits when in animal models.

The mouse IAKA model provides a strong analogue to the neuropathology of human MTLE. In Lévesque and Avoli's review of the model, it was concluded that intra-amygdaloid injections would produce both the behavioral seizures and neuropathological lesions similar to those seen in humans (2013; Araki et al., 2002). Histological analysis in the mouse model continues to be consistent with preferential unilateral neuron loss in the CA1 region of the hippocampus and prominent gliosis (Araki et al., 2002; Mouri et al. 2008). The consistency in pathology, both behavioral and histological, further justify the use of the IAKA model for translational purposes. Similar to behavioral aspects of MTLE in humans, the epileptic mice in this model do not always present behavioral or secondarily generalized seizures (the latter often described as tonic-clonic seizures). Seizures in the IAKA mouse model are highly stereotyped with subdued behavioral phenotypes, and many of the seizures being focal, resulting in a high frequency of subclinical electrographic seizures (Rattka et al. 2013, Silva et al. 2016, Tanaka et al. 2010). The uniformity of the seizure phenotype across different experiments in the IAKA model is absolutely crucial to the present study in order to establish a model which allows for a focus on refining the techniques and methodologies used to achieve the goal of sleep circuit manipulation as a seizure prevention mechanism. This proposed method of targeted manipulations of these circuits to control seizures has yet to be attempted.

Monitoring of seizures is heavily reliant on electroencephalographic (EEG) data, coupled with a clinical diagnosis of seizures (Engel, 2013). In this mouse model of MTLE, many seizures do not have a clear behavioral accompaniment, which makes EEG recordings critical for seizure analysis (Mouri et al., 2008). Kadam et al. (2017) provided a detailed review on the use of EEG technology for rodent models in order to ensure strong EEG signal with a high signal to noise ratio. New technology emerging from our lab (Zhu et al., 2020) has refined the process of creating a headcap for long-term intracranial EEG recording, allowing for rapid implantation for studying larger cohorts of mice, that is highly consistent between mice.

The establishment of a strong model and subsequent investigation into proposed manipulation of the sleep circuits aims to close an extensive gap in the literature and develop a new potential therapy, respectively. Much of the established sleep and epilepsy research is limited to showing, observationally, that there is a strong relationship between sleep and epilepsy, but there are almost no studies of mechanism and brain circuits in this context. Notable exceptions have examined orexin and crude basal forebrain lesions (Silveira et al., 2000), but have not extended to circuit-based studies with specifically identified cellular populations and targets, as is the future goal of this research. The development of novel treatments with chemogenetic manipulations of sleep-wake circuits, and normalization of sleep-wake behavior in the IAKA model of MTLE is expected to treat seizures with minimal side effects and potentially alleviate other comorbidities as well, thus increasing quality of life for TLE patients.

Methods

Mice. Mice (8-31 weeks old, N = 28) with a C57BL/6 J background of either sex were used in accordance with the Emory Institutional Animal Care and Use Committee. Mice were a transgenic VGAT-CRE strain, obtained from Jackson Labs (Stock No. 028862) and bred in our

own animal facility overseen by Emory Department of Animal Resources (DAR). Mice are provided with food, water, and bedding as well as a 12-hour light-dark cycle (7am-7pm) when housed in the Emory DAR facility. Breeding procedures included backcrossing and introduction of new mice for breeding purposes every 5 generations. For genotyping the animals, a DNA sample was obtained via ear punch and confirmed using PCR as per Jackson Lab's protocol.

Surgery. Mice were implanted with headsets, as per Zhu et al. (2020), with the addition of a microinjection guide cannula (5mm, cat# C315GMN/SPC, Plastics1, Roanoke, VA) and a dummy cannula (5mm, cat#C315FDMN/SPC, Plastics1, Roanoke, VA) targeting the basolateral amygdala. The addition of the cannulae requires using the stereotaxic frame to drill an additional hole in the skull (bregma -2.75, lateral -0.94) and a custom-made adaptor which enables to use of the stereotaxic frame to slowly lower the guide cannula until the base of the cannula pedestal is in contact with the top of the headset. Then, the base of the pedestal is glued to the top of the headset prior to cutting away the adaptor from the guide cannula. Following the surgical procedure, mice were kept warm as their anesthetic wore off prior to restoration of the righting reflex and returning to their home cages. Upon return to their home cage, mice recovered for 3-5 days prior to 48 hours of habituation to the recording environment and commutator.

Mouse Recording. Mice were singly housed in recording barrels (130 oz. clear acrylic container, Oggi Corporation, Anaheim, CA) equipped with drill holes for a water bottle (Choco Nose H125, Haywood, CA, one steel ball removed, affixed with 3d-printed adaptor, <https://3dprint.nih.gov/discover/3DPX-011069>) and in the center of the lid for the commutator (plate 3D filament printed to hold Pinnacle Commutator and a 1 megapixel day/night dome USP

security camera, ELP, Shenzhen, China, <https://3dprint.nih.gov/discover/3DPX-011070>). The mice are also supplied with food (Purina rodent chow), water, and nesting materials as needed and are kept in a temperature-controlled room with a 7am-7pm light-dark cycle. Video, EEG (ECog and hippocampal field potential), and EMG were recorded continuously throughout the experiment, which is approximately 4 weeks. Pre-amplifying headsets (8406-SE31 M, 100x gain, Pinnacle Technologies, KS) were used with a commutator (model 8408, Pinnacle Technologies, KS) and analogue breakout boxes (8443-PWR, Pinnacle Technologies, KS) with additional gain and digitization (Power 1401, CED, Cambridge, UK). Synchronized video and EEG/EMG files were saved as 12-hour blocks with Spike2 (v9.10, CED, Cambridge, UK), and transferred to a laboratory server for later analysis using a PowerShell script.

Kainic Acid Injection. Following the habituation period and 7 days of 24 hour baseline recording, mice underwent injection of either saline or kainic acid into the basolateral amygdala 5-7 hours after lights on (0.3 μ g in 200 nL over 5 minutes). Injections required the removal of the dummy cannula and placement of the internal cannula (1.75mm projection, cat# C315IMN/SPC, Plastics1, Roanoke, VA) Video-EEG recordings are continuous during the IAKA injection. Mice were injected with diazepam (5mg/kg, i.p.) 40 minutes after the end of the kainic acid infusion. Recording then continued for another three weeks. Given the high variability in the number of seizures in injected mice, as well as a mortality rate of 27.87% (N=61, 16.39% died during SE, 11.48% died later in the experiment), there were 17 IAKA mice, 4 IAKA injected controls who did not have seizures, and 7 saline injected controls included in these data as they survived the entire three week recording period following injections, had on-target injections, and had successful EEG recordings for the duration of the experiment.

Perfusion and Histology. At the end of the three-week experiment, mice were disconnected from the recording apparatus, given Euthasol (pentobarbital, 150 mg/kg, i.p.) to induce a state of deep anesthesia and then transcardially perfused with saline followed by 10% formalin in neutral phosphate-buffered saline (PBS). Brains were extracted and post-fixed overnight in the 10% formalin prior to being placed in PBS sucrose (30%) for a minimum of 24 hours. Brains were then mounted on a freezing microtome (American Optical Corp, Buffalo, NY) in OCT and cut into 35 μ m coronal sections. Free floating sections then underwent immunohistochemical staining with a diaminobenzidine (DAB) chromogenic step, for neuronal nuclear protein (M-NeuN, 1:1000 μ L, lot #3468221, MilliporeSigma, Burlington, MA) with NiCo enhancement, then glial fibrillary acid protein (Rb-GFAP, 1:20k μ L, lot #20047046, Agilent Dako, Denmark) before being mounted on slides and coverslipped for microscopy.

Data Analysis. Sleep and seizure were scored manually using Spike2 (v9.10, CED, Cambridge, UK). Precise seizure onsets and ends were marked, and were scored when high amplitude, rhythmic evolving activity was seen in the record, lasting 5 seconds or longer. Sleep-wake scoring was completed using the convention established by Rechtschaffen and Kales (1968), per standard rodent scoring criteria, using 20 second epochs of wake, rapid eye movement (REM) or non-rapid eye movement (NREM) sleep, with the addition of seizure and postictal generalized EEG suppression (PGES) states. Additionally, using the seizure scores as a guide for onset time, seizures are to be scored behaviorally using the video data and following the traditional Racine scale scoring criteria.

Summary Data and Statistical Analysis. Data was compiled and analyzed using descriptive statistical tests, 2-way ANOVA, linear regression and correlation via Prism.

Results

Epilepsy and Seizures. Of 21 mice injected with intra-amygdala kainic acid, 17 developed seizures. Seizures may present behaviorally with a variety of characteristics as depicted via the Racine scoring criteria. A score of 1 presents as immobility and freezing, a score of 2 presents as forelimb and/or tail extension and rigid posture, a score of 3 presents as head-bobbing and other repetitive movements, a score of 4 presents as rearing and falling, a score of 5 presents as continuous rearing and falling, and a score of 6 presents as severe tonic-clonic seizures (Jimenez-Pacheco et al. 2013). Not all seizures will have a behavioral component, however, as lower-grade seizures will often only have the electrographic component.

Sleep Disruption. The proportions of average time spent per state—wake, NREM, REM, and ictal period—per hour of the final 3 days were used for analysis of sleep-wake patterns across all mice (N=28). Upon initial plotting of these averages for each state as seen in Figure 1, it is noticeable that there is very little difference between the data for the IAKA (without seizure) group and the saline control. However, due to the small sample size for the IAKA (without seizure) group (N=4), the data is severely underpowered thereby preventing any substantial claims regarding that population at the present. For the following analyses, the IAKA (without seizure) group has been omitted in order to focus on the differences between the saline control and IAKA mice.

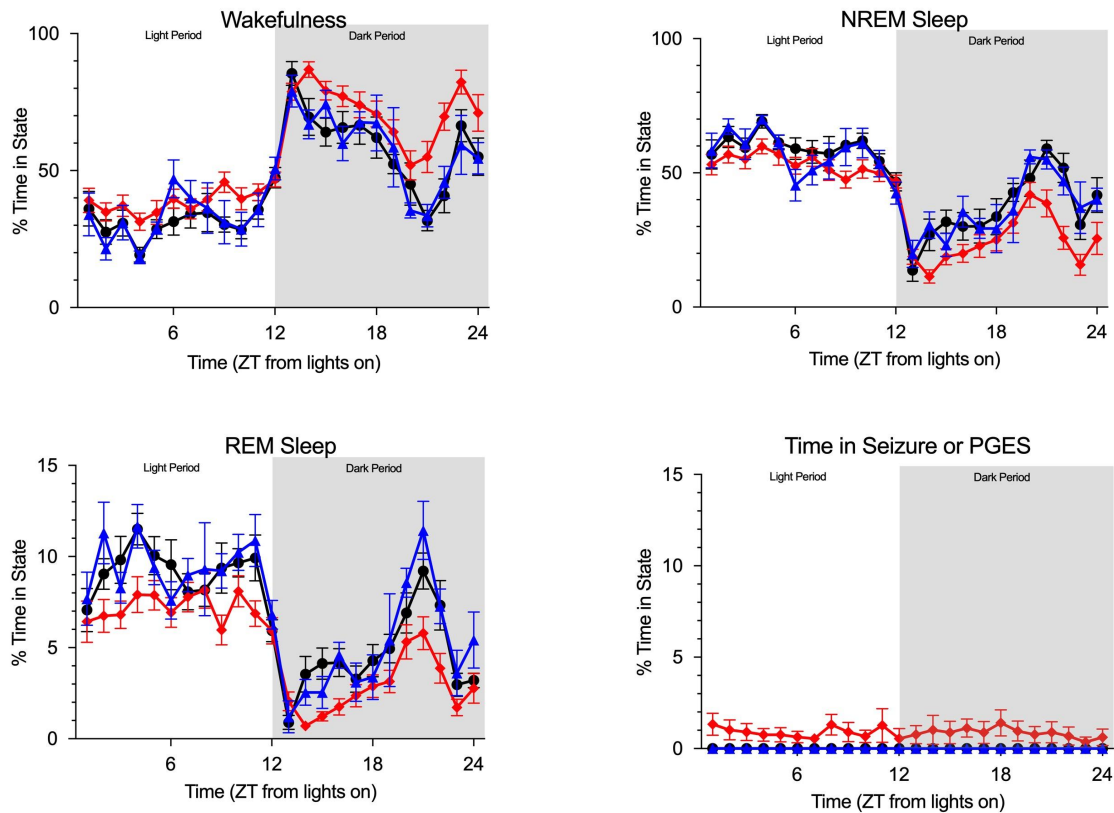


Figure 1. Time spent in each sleep state, as well as in a state of postictal generalized EEG suppression (PGES), per light-dark period. Data was averaged every hour for each mouse over the final 72 hours of the experiment. Black = Control (saline), Red = IAKA (with seizures), Blue = IAKA (no seizures). IAKA mice had a significant difference in each of the four states compared to the saline control mice, $p < 0.0001$.

Treatment had a statistically significant effect on the average time spent in wake across the final three days of the experiment, $F(22,506)=6.364$, $p < 0.0001$. Treatment had a statistically significant effect on the average time spent in NREM sleep across the final three days of the experiment, $F(22,506)=7.572$, $p < 0.0001$. Treatment had a statistically significant effect on the average time spent in REM sleep across the final three days of the experiment, $F(22,506) = 6.316$, $p < 0.0001$. Treatment had a statistically significant effect on the average time spent in the combined ictal and postictal states over the final three days of the experiment, $F(22,506)=74.42$, $p < 0.0001$. Despite the saline group's time spent in the ictal period being zero, the overall time in these states was very small even in mice with seizures.

Distribution of Seizures. Proportion of seizures by origin state was calculated for each IAKA mouse with reported seizures. Total number of seizures across all mice in this group (N=17) was 728 seizures, with 33.79% of seizures originating from wake, 38.30% of seizures originating from NREM, 9.17% of seizures originating from REM, and 18.74% of seizures originating from the postictal state.

Continuing to use the final 72 hours of the experiment for analyses of sleep-wake patterns, the average amount of time spent in each state was calculated for each IAKA mouse prior to being compiled into a summary data table. 55.31% of the time was spent in wake, 38.86% of the time was spent in NREM, 4.96% of time was spent in REM, and 0.87% of the time was spent in the postictal state.

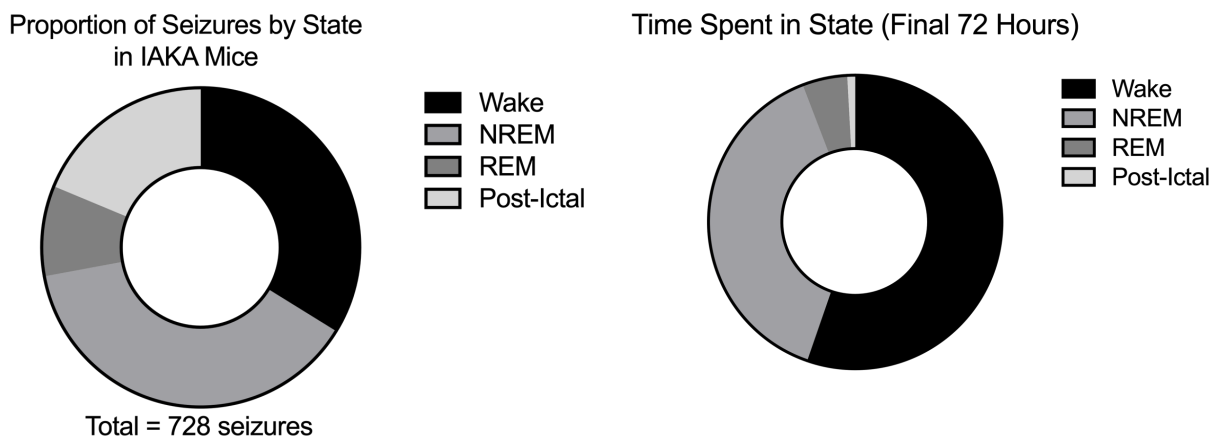


Figure 2. Proportions of seizures by state in IAKA mice throughout the 3 week experiment compared to the distribution of time spent in each state over the final 72 hours.

The number of electrographic seizures were plotted against the percent wakefulness over the final 72 hours of the experiment for the IAKA mice. Seizure burden did not have a

statistically significant positive correlation with percent wakefulness, $r=-0.3195$, $p=0.2655$. This correlation can be seen plotted as a linear regression in Figure 3.

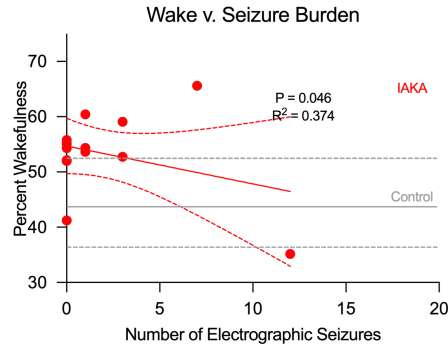


Figure 3. Percent wake vs. seizure burden. Number of identified electrographic seizures plotted against the amount of time each individual animal spent in the wake state. Outliers were omitted from the regression.

Duration of Seizures. Data was compiled from the light and dark periods to account for the entire experimental period for all IAKA mice with reported seizures (N=17). Seizure duration was quantified by the mean duration of seizures per prior state across wake, NREM, REM, and postictal states. Two-way ANOVA results for seizure duration by onset state identify a significant effect of state on average seizure duration for IAKA mice, $F(3,6)=11.99$, $p=0.0060$. Tukey's multiple comparisons test yields a statistically significant difference for the NREM-Postictal interaction ($p=0.0061$) and REM-Postictal interaction ($p=0.0130$). All other comparisons yielded results that were not statistically significant. However, it is important to note that the overall time spent in the postictal phase was significantly less than in any of the other states, therefore results are likely underpowered. Overall comparison of seizure duration by onset state can be seen in Figure 4.

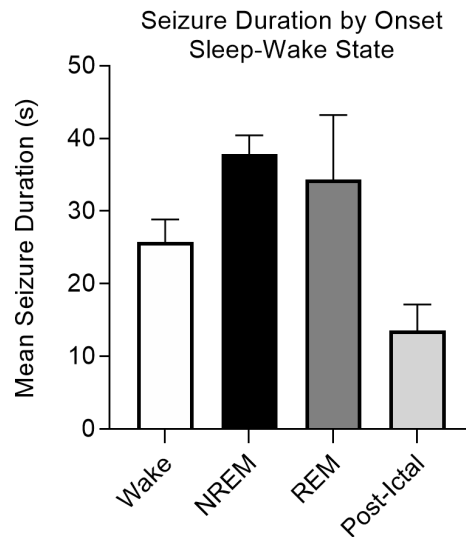


Figure 4. Seizure duration by onset sleep-wake state over the duration of the experiment. The difference between NREM and Postictal ($p=0.0061$) and REM and Postictal ($p=0.0130$) were statistically significant.

Histopathology. Preliminary histopathological analyses identify damage which extends beyond the basolateral amygdala, likely as a result of diffusion of the kainic acid following the initial injection. Structures surrounding the amygdala including the piriform cortex, globus pallidus, striatum, caudate, and putamen appear to be damaged by the injection as characterized by neuron loss and reactive gliosis. Additionally, animals who had seizures exhibited neuron loss in the CA1 and CA3 regions of the hippocampus, while CA2 and the dentate gyrus were spared. Gliosis in the hippocampus often accompanied the cell loss in CA1 and CA3.

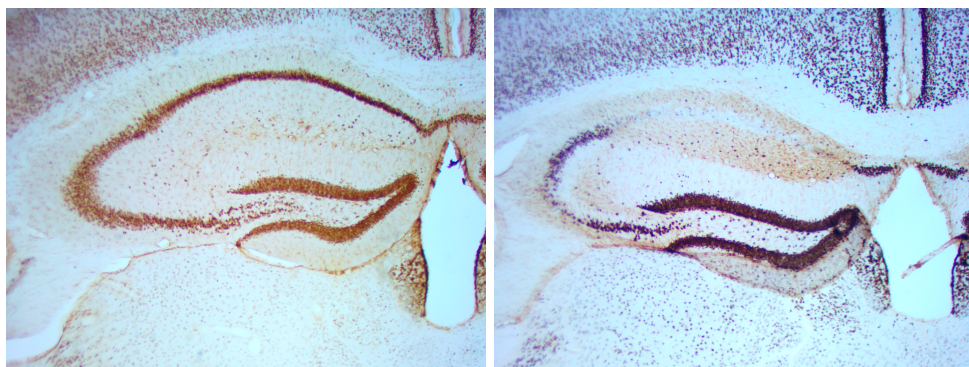


Figure 5. Histopathology of IAKA injection. Mouse without seizures and intact hippocampus is on the left for comparison. IAKA mice showed bilateral hippocampus damage with severe neuron loss in CA1 on both sides. Neuron loss is also seen in the CA3 region, which is more severe on the side ipsilateral to the injection.

Discussion

Key Findings. It was shown that sleep was significantly decreased (by about 10%) in both the light and dark cycles for mice that received the IAKA injection. The decreased and fragmented sleep was coupled with spontaneous seizures, however, longer and disproportionately frequent seizures occurred when the onset was in wake. Additionally, transitions from sleep to wake were observed at a much higher frequency in IAKA mice, but further analysis is needed to determine significance. These findings mirror documented human sleep disorders in epilepsy, specifically with prominent insomnia and sleep disruption. Understanding the patterns of sleep disruption allows for not only further justification of the mouse IAKA model of MTLE, but also shifts the focus towards treating the sleep disorders often experienced by MTLE patients. Furthermore, the histopathology as seen in this experiment is consistent with previous literature in the rodent IAKA model, sparing CA2 and the dentate gyrus while prominent cell loss occurs in CA1 and CA3.

Limitations. The intraamygdala kainic acid (IAKA) model is only one model of seizure induction, and so it would be beneficial to replicate this experiment with another model of temporal lobe epilepsy to ensure consistency in our findings. The results obtained in the present study pose the question of whether sleep disruption is due to seizures and clusters of seizures, or whether it is a feature of the epilepsy or interictal phenomena. Furthermore, as a result of preliminary histological analyses, placement of the cannula needs to be refined in order to be more on target.

Perhaps the most prominent limitation is understanding causality as it relates to sleep disruption and seizure occurrence - it seems likely that sleep loss can increase seizures, which may in turn disrupt sleep. The same question arises when examining the sleep disruption seen in this rodent model and in patients; is sleep worse immediately following seizures or is sleep consistently poor despite the clustered seizure activity. Furthermore, the lack of information regarding direction of causality poses the question of the extent to which the sleep disruptions influence depression, anxiety, memory deficits and attentional difficulties. In these studies to establish a model of human sleep disruption as a comorbidity of epilepsy, we did not examine the effects of treatment, and may compare these with novel therapies in future studies.

Future Directions. The nature of this study is establishing a sleep-based model of KA induced MTLE in rodents, allowing for a variety of future directions to stem from the present research. With the currently available data, there are additional analyses that have yet to be completed. These include comparing the current VGAT-CRE mice - known to have a lower threshold for electrical kindling - to wild type C57BL/6 J mice, sleep fragmentation analysis focusing on severity compared to controls as well as the evolution over the three week recording period, and

evolution of wake versus seizure burden across the three weeks. Additionally, we aim to replicate the findings from the IAKA model with another model, such as electrical stimulation kindling. Using the present project as a starting point, there are three main proposed future directions in which the Pedersen lab aims to accomplish in succession to this project: chemogenetic manipulation of the subcortical sleep-wake circuits, behavior studies with a focus on changes in memory, and machine and deep learning for automating data analysis.

The proposed manipulation of the epileptogenic circuits as they relate to sleep-wake in the IAKA mouse model would serve to bridge the gap in the literature using established techniques, such as chemogenetics, in conjunction with developing a novel method of treatment for MTLE patients, and form the basis for studies that examine the role of sleep disruption in exacerbations of other epilepsy comorbidities. More specifically, this would entail using a CRE-recombinant dependent vector to transduce excitatory muscarinic receptors in appropriately targeted neurons, consistent with Pedersen et al. (2017). This targeted manipulation will aim to promote sleep or wake, depending on which structure is being actively targeted with the vector, by activation and inhibition of sleep-wake circuits.

Successful chemogenetic manipulation of sleep circuits to normalize sleep-wake behavior in epileptic mice has a translational potential and significance with its intent to develop novel treatments for patients. This could range from rationalizing sleep interventions to vector-based technologies for chemogenetics and related therapies. Utilizing a chemogenetic approach to normalize sleep behavior would provide temporal lobe epilepsy patients with a novel treatment option that has a potentially lower side-effect profile. Additionally, if shown to be a viable treatment option, the improved treatment of seizures can provide relief from the sleep disruptions which often decrease quality of life for many patients and may contribute to other comorbidities.

Taking a behavioral approach in future studies is two-fold; the goal is to investigate changes in memory as a result of KA-induced damage to the hippocampus, and assess the effect of the aforementioned chemogenetic treatment, assuming it yields the intended results. Again, this combination could be the foundation of a novel treatment mechanism for epilepsy in addition to inquiring about the relationship between memory changes and sleep in individuals with epilepsy.

Also underway is the use of deep learning to score the voluminous data collected by our method. Presently, the sleep and seizure data is scored manually in the Spike2 software with the assistance of customized scripts, however this is not sustainable long-term. A major obstacle in the development of deep learning methods has been the variability in the abnormal EEG signal of KA-injected mice. This makes the otherwise straightforward scoring of sleep and seizure, individually, rather difficult when combined.

Conclusion

The present study opens up a new perspective on the IAKA rodent model of TLE with a focus on the effects of the seizures on sleep. About one million epilepsy patients, not limited to (M)TLE patients, have disordered sleep and many of the commonly recognized comorbidities of epilepsy are worsened by disrupted sleep. Working to develop a better understanding of how sleep is disrupted in mice injected with kainic acid is establishing a foundation for how to better approach potential treatments for patients with MTLE. Historically, there is not much available on the effects of sleep disruption in patients with temporal lobe epilepsy, just that they are more likely to experience insomnia and other forms of fragmented sleep. With the notable spiral effects that lack of sleep can have on memory and cognition combined with the roughly 33% of

patients who do not respond well to currently available medications (Granata et al. 2009, Kwan & Brodie 2000), developing a novel treatment that focuses on normalizing sleep rather than stopping seizures can ideally lead to better treatment outcomes with a more mild side-effect profile.

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